

# $\beta$ -Amyloid Plaques Induce Neuritic Dystrophy of Nitric Oxide-Producing Neurons in a Transgenic Mouse Model of Alzheimer's Disease

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**A causative role for nitric oxide has been postulated in a number of neurodegenerative diseases. Using histochemical and immunohistochemical methods, we examined the effect of  $\beta$ -amyloid plaques on nitric oxide-producing cells in transgenic mice which overexpress a mutant human amyloid precursor protein (APP). In 14-month-old animals, nitric oxide synthase (NOS)-positive dystrophic neurites were observed frequently in the cerebral cortex and hippocampus of all of 16 plaque-bearing transgenic animals and in none of 16 wild-type animals. Double labeling of NOS and  $\beta$ -amyloid revealed that 90% of  $\beta$ -amyloid plaques were associated with NOS-containing dystrophic neurites. In 7-month-old animals,  $\beta$ -amyloid plaques were very rare, but those present were frequently associated with NOS-positive neuritic dystrophy. We conclude that  $\beta$ -amyloid plaques induce neuritic dystrophy in cortical neurons containing NOS in this model of AD, and hypothesize that this finding may be relevant to the mechanism of  $\beta$ -amyloid neurotoxicity in human AD.**

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**Key Words:** stress; oxidative; free radicals; disease models; animal.

## INTRODUCTION/BACKGROUND

The pathophysiology of neurodegeneration in Alzheimer's disease (AD) remains poorly understood. A model based on a neurotoxic effect of  $\beta$ -amyloid ("the amyloid cascade hypothesis") is supported by the findings that several genes implicated in AD are associated with increased levels of  $\beta$ -amyloid (36, 44, 58) and by direct evidence for  $\beta$ -amyloid neurotoxicity *in vitro* (7, 54). Neurotoxicity in the living animal has not been repro-

ducibly demonstrated, although evidence that neurotoxicity *in vivo* is dependent on the age of the animal (14) and the state of  $\beta$ -amyloid aggregation (30) may account for some of the variability in findings to date.

Several transgenic mouse strains have been developed as models of AD or of cerebral  $\beta$ -amyloidosis (13, 20, 21, 39, 48). All of these strains develop cerebral  $\beta$ -amyloid plaques and some have been shown to have dystrophic neurites associated with plaques. However, none have been shown to develop neurofibrillary tangles, and only one strain has exhibited neuronal loss in brain regions affected by plaques (4). In the present study, we use a mouse strain which overexpresses a mutant form of the human  $\beta$ -amyloid precursor protein (huAPP) (21). This strain has been shown to exhibit behavioral abnormalities (21) and impaired long-term potentiation (6) which correlate with the appearance of cortical and hippocampal  $\beta$ -amyloid plaques. The structural basis of the neurologic dysfunction is unknown, since there is no evidence for neuronal death or synaptic loss (25). The best evidence for neuronal damage in this animal model is the finding that some plaques are surrounded by dystrophic neurites, visualized by either Gallyas silver staining or immunohistochemistry for neurofilament or tau proteins (21, 25).

Several mechanisms for  $\beta$ -amyloid neurotoxicity have been proposed. There is abundant evidence for a mechanism involving free radicals and oxidative stress. *In vitro* studies demonstrate that antioxidants can prevent  $\beta$ -amyloid-associated neurotoxicity in culture (29, 40, 53, 55, 56). In addition, brains from patients with AD harbor elevated levels of oxidative end products of lipids, proteins, and nucleic acids (31) when compared to age-matched controls. Finally, antioxidant therapy with vitamin E has been shown to slow the rate of AD progression (42).

A second mechanism implicated in  $\beta$ -amyloid neurotoxicity and in AD is inflammation (10). An apparent inflammatory response to  $\beta$ -amyloid is observed in the

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AD brain, in the form of activated microglia and the presence of a number of inflammatory mediators in the vicinity of the  $\beta$ -amyloid plaque (10). Moreover, epidemiologic studies consistently find that users of anti-inflammatory medicines have a reduced incidence of AD (33). Clinical trials of anti-inflammatory agents for AD are currently underway.

There is evidence for the involvement of both inflammatory and oxidative stress mechanisms in the transgenic mouse strain used in these studies. For example, oxidative end products have been described in the brains of mice which have reached plaque-bearing age (38, 46), and activated microglia have been observed surrounding the  $\beta$ -amyloid plaques (12).

We tested the hypothesis that nitric oxide mediates inflammation and oxidative stress associated with cerebral  $\beta$ -amyloidosis in these animals. Nitric oxide has several potential roles in the brain, as a neurotransmitter, as a potential source of free radicals and oxidative stress, and as a potential mediator of inflammation (5). Nitric oxide is synthesized by the enzyme nitric oxide synthase (NOS), for which three genes exist: neuronal NOS (nNOS, NOS-I, or bNOS), inducible NOS (iNOS, NOS-II, or macNOS), and endothelial NOS (eNOS, NOS-III) (5). Nitric oxide produced by iNOS in microglial cells is involved in inflammatory processes, and we hypothesized that iNOS would be up-regulated in the vicinity of plaques.

We found that NOS activity is in fact increased in the vicinity of cerebral  $\beta$ -amyloid plaques, but contrary to our expectations the cellular origin appears to be neuronal and the enzyme responsible for the increased activity appears to be neuronal NOS. Some dystrophic change is seen even with very early plaques, though the change appears to be more robust in older animals.

## MATERIALS AND METHODS

### *Animals*

Transgenic mice, a generous gift from Dr. Karen Hsiao (Mayo Clinic), were bred with C57B6 wild-type mice and several litters were genotyped and weaned. Forty-one female mice were used in this study: 4 wild-type and 5 transgenic huAPP at 7 months and 16 wild-type and 16 transgenic huAPP at 14 months. The animals were sacrificed by cervical dislocation. All experiments were approved by the Oregon Health Sciences University Institutional Animal Care and Use Committee in accordance with NIH guidelines.

### *Tissue Preparation*

Mouse brains were rapidly removed. The left hemisphere was frozen for biochemical analysis. The right hemisphere was immersion fixed in 4% formalin in phosphate-buffered saline (PBS), with gentle shaking

at 4°C for 12–18 h. The right hemisphere was then rinsed in PBS and cryoprotected in 30% sucrose in PBS, and 40- $\mu$ m frozen sections were cut on a sledge microtome.

### *Histochemistry and Immunohistochemistry*

**Histochemistry.** Histochemical stain for diaphorase (43) was completed on the day of sectioning, as enzyme activity decays over several days. Enzyme activity is undetectable in tissue which is heavily fixed or paraffin-embedded. Sections were incubated for 4 h with shaking at 37°C in PBS containing 0.5 mg/ml of  $\beta$ -NADPH (Sigma-Aldrich), 0.1 mg/ml of 4-nitroblue tetrazolium chloride (NBT, Boehringer-Mannheim), and 0.1% Triton X-100. Sections were then rinsed with PBS and mounted in glycerol or maintained in PBS for subsequent immunohistochemistry.

**Thioflavin S.** Sections were mounted and dried on subbed slides. Slides were then incubated in 1% thioflavin S (Sigma-Aldrich) in water for 10 min at room temperature. Slides were then dehydrated in an alcohol series and coverslipped with Permount.

**Immunohistochemistry for  $\beta$ -amyloid, GFAP, MAC-1, and nNOS.** All incubations were carried out at room temperature. Sections were incubated in a blocking solution of Tris-buffered saline (TBS, 100 mM Tris, 150 mM NaCl, pH 8.0) containing 2 mg/ml bovine serum albumin (BSA), 2% horse serum, and 0.5% Triton X-100 for 2 h with agitation. The blocking buffer was then replaced with primary antibody diluted in the same solution.

Rabbit polyclonal antibodies directed against  $\beta$ -amyloid (Sigma-Aldrich, 1:2000), GFAP (Biomedical Technologies, 1:1000), and nNOS (Biomol, 1:1000); and rat monoclonal antibodies directed against somatostatin (Accurate, 1:50) and MAC-1 (Serotec, 1:500) were used. Sections were incubated in primary antibody overnight, for 2 h with a biotinylated secondary antibody (Vector, 1:200), for 2 h with an avidin-linked peroxidase complex (ABC, Vector), and developed with diaminobenzidine (DAB) in PBS. Sections were washed and mounted in glycerol.

The specificity of the anti-nNOS staining was tested by incubation of the nNOS antibody (1:1000) with the peptide used to produce the antibody (Biomol) at 50  $\mu$ g/ml before the tissue was stained. Sections incubated in primary antibody with and without preabsorption were processed in parallel.

**Immunofluorescent double labeling of nNOS and somatostatin.** Twenty micron thick sections from transgenic huAPP mice were incubated overnight in primary antibodies for immunohistochemistry and then incubated in a biotinylated anti-rat antibody for 2 h, as above. Sections were then incubated with an avidin-linked-rhodamine complex (Molecular Probes, 1:1000)

and an FITC-labeled anti-rabbit antibody (Cappel, 1:500) for 2 h and mounted in Vectashield (Vector).

### Analysis

Quantitative analysis of total cerebral area occupied by  $\beta$ -amyloid,  $\beta$ -amyloid plaque number, plaque-associated NOS abnormalities, and plaque-independent NOS abnormalities was carried out with representative frontal cortex sections.

Coronal sections of three of the 14-month-old transgenic mice were analyzed using a computer-driven microscope stage and the Stereo-investigator software package. Representative sections of frontal cortex, at the level of the striatum, anterior to the fornix, were selected for analysis. The analyzed sections were stained histochemically for diaphorase and immunohistochemically for  $\beta$ -amyloid as described above. The analysis was confined to cerebral cortex because hippocampus had fewer  $\beta$ -amyloid plaques. Under low magnification (25 $\times$ ) the perimeters of  $\beta$ -amyloid plaques were traced, and computer software calculated the area of each plaque as well as the total area of all  $\beta$ -amyloid plaques. Each plaque thus identified was subsequently examined under higher power (100 $\times$ ) and characterized according to the degree of associated diaphorase-positive neuritic dystrophy on a scale of 0–4. A grade of 0 describes a plaque with no associated diaphorase-positive neuritic dystrophy. A grade of 1 describes a plaque with subtle neuritic dystrophy, such that the involved neurites are not greater than approximately twice the caliber of a normal axon. Grade 2 describes a plaque with obvious diaphorase-positive neuritic dystrophy with the involved axons still recognizable as linear structures. Grade 3 describes moderately severe neuritic dystrophy, with axons having a bulbous appearance and grade 4 describes severe neuritic dystrophy, with several bulbous axonal swellings per plaque (Fig. 3). The total cortical gray matter was traced and the area calculated. The percentage of cortical area occupied by  $\beta$ -amyloid plaques, the density of  $\beta$ -amyloid plaques per square millimeter, the number and percentage of total plaques in each category of diaphorase abnormality, the number of each diaphorase-defined plaque type per square millimeter, and the mean plaque size for each category of diaphorase abnormality were then determined. Diaphorase abnormalities which were not associated with plaques were also counted. Values for these parameters were expressed as means for the three animals examined. Coronal sections of five 7-month-old transgenic mice were also examined.  $\beta$ -amyloid plaques were identified and graded in terms of neuritic dystrophy in the same manner.

## RESULTS

### 1. Distribution of $\beta$ -Amyloid Plaques

In the 7-month-old mice,  $\beta$ -amyloid plaques were quite rare in the cerebral cortex (0–2 per section, 36 in the 45 sections examined), and only one plaque was seen in hippocampus. Plaques occupied 0.01% of the cortical area examined in the 7-month-old animals.

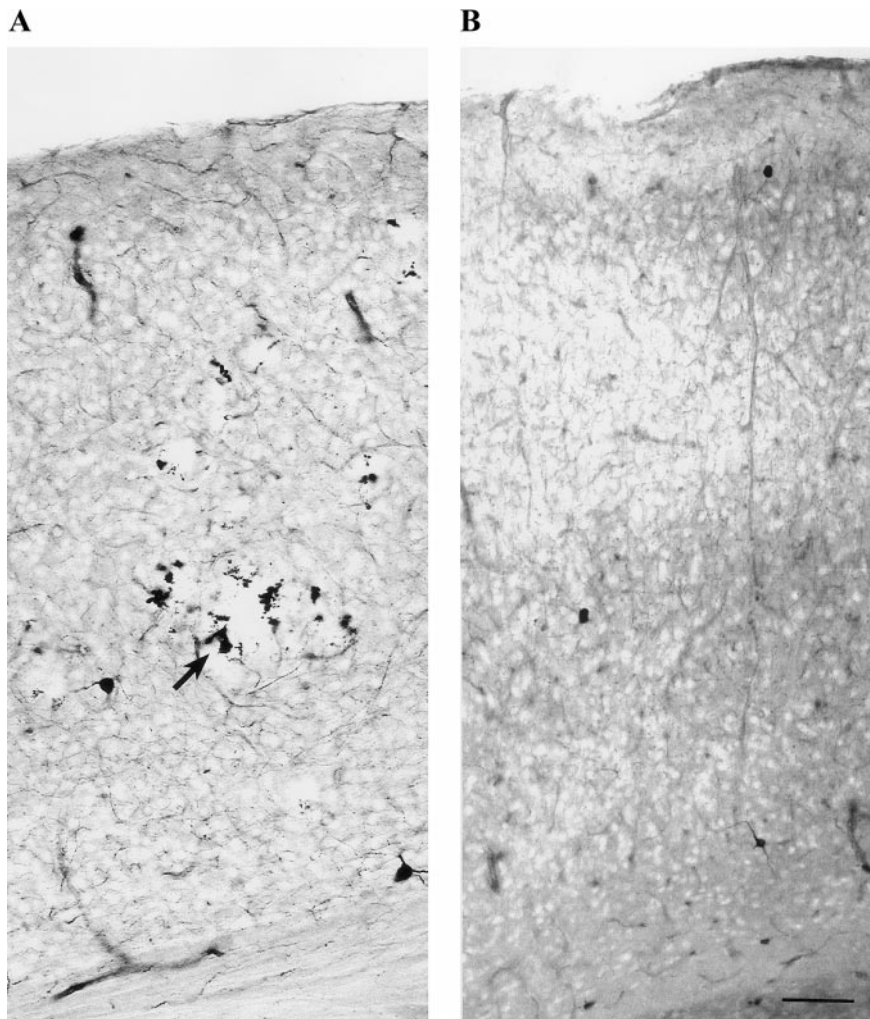
In the 14-month-old transgenic mice,  $\beta$ -amyloid plaques were abundant in every section of cerebral cortex, occasional in hippocampus, and absent in the subcortical areas. Plaques appeared to be homogeneously distributed throughout the cortex, from frontal to occipital pole, with no region of predominance in this plane. In the three mice analyzed,  $\beta$ -amyloid plaques occupied  $1.4 \pm 0.3\%$  of the total cortical area.

### 2. Diaphorase Histochemistry

Serial coronal sections, spaced at 480- $\mu$ m intervals through the brains of five 7-month-old huAPP mice, sixteen 14-month-old huAPP mice, and sixteen 14-month-old wild-type mice, were stained histochemically for diaphorase. While NADPH diaphorase activity in the brain has been shown to be identical with nitric oxide synthase activity (8), the histochemical method does not distinguish between different NOS isoforms.

In all of the 14-month-old transgenic huAPP mice, swollen diaphorase-positive structures which appeared to be dystrophic neurites were seen in the cerebral cortex (Fig. 1). None of these structures were seen in any of the 14-month-old wild-type animals. In some sections, these dystrophic structures were obviously contiguous with normal caliber, diaphorase-positive axons coursing through the cerebral cortex. These dystrophic, diaphorase-positive structures were abundant in the cerebral cortex of plaque-bearing mice, but relatively rare in the hippocampus. In the 7-month-old transgenic mice, diaphorase-positive dystrophic structures were rare, with a morphology similar to the less robust structures in the 14-month-old mice. None of the dystrophic diaphorase-positive structures in the 7 month old mice reached the size and complexity of the most robust dystrophy in the older mice.

Normal-appearing neuronal cell bodies and fibers were labeled in the cerebral cortex of all animals examined. Blood vessels were also labeled, but astrocytes and microglia were not. Some normal-appearing neurons, axons, and blood vessels were also seen in the 14-month-old transgenic animals. Microglia were not labeled in the vicinity of the plaques or elsewhere in these brains.



**FIG. 1.** Diaphorase stain, a nonspecific histochemical stain for nitric oxide synthase, in 14-month-old wild-type and transgenic huAPP mice. Neuronal cell bodies, axons, and blood vessels are labeled in both wild-type and transgenic animals. In the transgenic (A) but not the wild-type animal (B), swollen, dystrophic-appearing structures (arrow) are also labeled throughout the cerebral cortex. Bar = 100  $\mu$ m.

### 3. Immunohistochemistry for Neuronal NOS and for Somatostatin

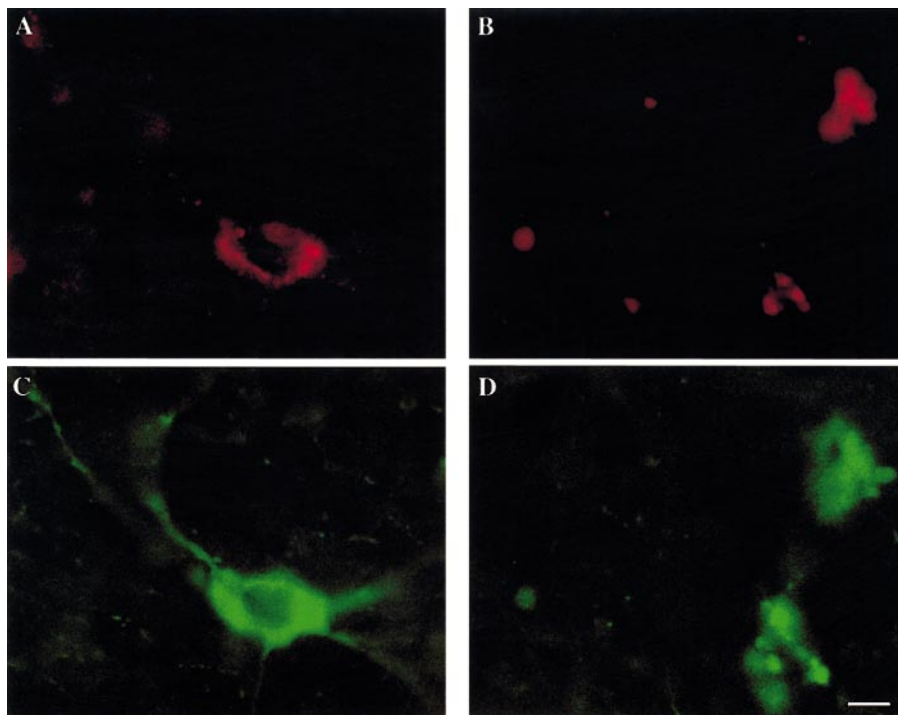
Neuronal cell bodies, axons, and clusters of dystrophic neurites, similar in appearance and density to the diaphorase-labeled structures, were seen with nNOS-specific immunohistochemistry (Fig. 2). In contrast to the diaphorase stain, this method did not label blood vessels. The neuronal and terminal labeling were completely abolished by preabsorption of the anti-nNOS antibody with nNOS peptide at 50  $\mu$ g/ml.

Immunohistochemistry for somatostatin, which is known to be colocalized with nNOS in cortical neurons (26–28), labeled both cortical neurons and dystrophic structures that appeared similar to those labeled by nNOS. Double labeling of sections with nNOS and somatostatin antibodies confirmed that nNOS and somatostatin are co-localized within the same neuronal cell bodies and dystrophic neurites (Fig. 2). Overall,

nNOS labeling was more intense than that for somatostatin, and over half of the nNOS-positive dystrophic neurites also contained somatostatin immunoreactivity. Only very few somatostatin-positive, nNOS-negative dystrophic neurites were observed. Interestingly, in some cases, somatostatin immunoreactivity was observed in only some, but not all branches of a dystrophic nNOS-positive neurite.

### 4. Double Labeling with NOS Histochemistry and $\beta$ -Amyloid Immunohistochemistry

The close association of  $\beta$ -amyloid plaques and dystrophic NOS-positive neurites was evident in sections from 14-month-old animals stained for both substances (Fig. 3). Ninety percent of the plaques (232 of 257 total analyzed) were associated with dystrophic NOS-positive neurites, although the degree of dystrophic change varied between plaques. There was a trend for larger



**FIG. 2.** Double staining of cerebral cortex of 14-month-old transgenic huAPP mouse for the neuropeptide, somatostatin (red, A, B) and neuronal nitric oxide synthase (nNOS; green, C, D) using immunofluorescence. Somatostatin and nNOS are localized in a neuronal cell body (A and C) and in dystrophic neurites (B and D). The nNOS labeling (C and D) was consistently more intense and somewhat more extensive than the somatostatin labeling (A and B). Bar = 7.5  $\mu$ m.

plaques to be associated with more severely dystrophic NOS-positive neurites (Table 1). Dystrophic diaphorase-positive neurites were extremely rare in areas not containing noticeable plaques. The topographic distribution of plaques in a representative coronal section of frontal cortex is shown in Fig. 4.

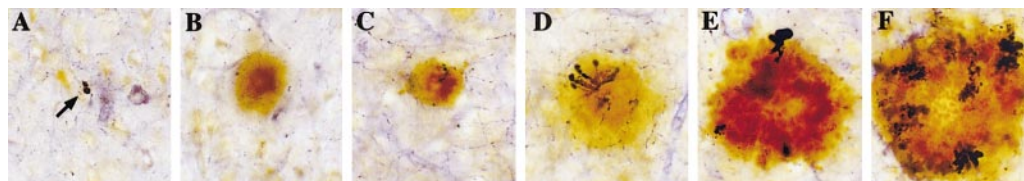
Thioflavin S staining, in combination with diaphorase histochemistry, confirmed that the  $\beta$ -amyloid plaques associated with the dystrophic neurites contained fibrillar  $\beta$ -amyloid (Fig. 5).

In order to determine if neuritic dystrophy is an early event relative to amyloid plaque formation, a group of younger animals was studied. In the 7-month-old animals, the plaques were so rare (0–2 per section) that the results cannot be presented on the same scale as those of the 14-month-old mice.

Among the 36  $\beta$ -amyloid plaques identified in the 7-month-old mice, 7 had no associated neuritic dystrophy, 21 were grade 1, 8 were grade 2, and none were grade 3 or 4.

##### 5. Double Labeling with NOS Histochemistry and GFAP/MAC-1 Immunohistochemistry

The area circumscribed around plaques by activated astrocytes (GFAP, Fig. 6A) was larger than the area occupied by  $\beta$ -amyloid and diaphorase activity. The labeling of microglia (MAC-1, Fig. 6B) was less robust and less extensive. Double staining with diaphorase and markers for astrocytes and microglia confirmed that these nonneuronal elements do not contribute fibers to the NOS-positive dystrophic structures.



**FIG. 3.** Double staining of cerebral cortex of 14-month-old transgenic huAPP mouse (A–F) using diaphorase histochemistry (blue) and  $\beta$ -amyloid immunohistochemistry (brown). The grading scheme for plaque-associated neuritic dystrophy is illustrated. (A) Dystrophic neurites without an associated plaque. (B) Grade 0,  $\beta$ -amyloid plaque without associated neuritic dystrophy. (C) Grade 1 neuritic dystrophy. (D) Grade 2 neuritic dystrophy. (E) Grade 3 neuritic dystrophy. (F) Grade 4 neuritic dystrophy. See text for details of grading system. Bar = 20  $\mu$ m.

**TABLE 1**Relative Numbers, Sizes, and Densities of  $\beta$ -Amyloid Plaques in 14-Month-Old Mouse

Plaque type	Plaques per mm <sup>2</sup>	Mean plaque size (in $\mu\text{m}^2$ )	Percentage tissue affected
0	0.650	1203	0.079
1	5.033	646	0.322
2	2.534	985	0.250
3	1.406	1935	0.270
4	0.705	6886	0.476

*Note.* The data are presented as the mean  $\pm$ SD for three animals. The numbers referring to plaque type correspond to the grading scheme illustrated in Fig. 3. The plaques were predominantly grades 1–3. However, there is a trend for larger plaques to be associated with higher grades of neuritic dystrophy.

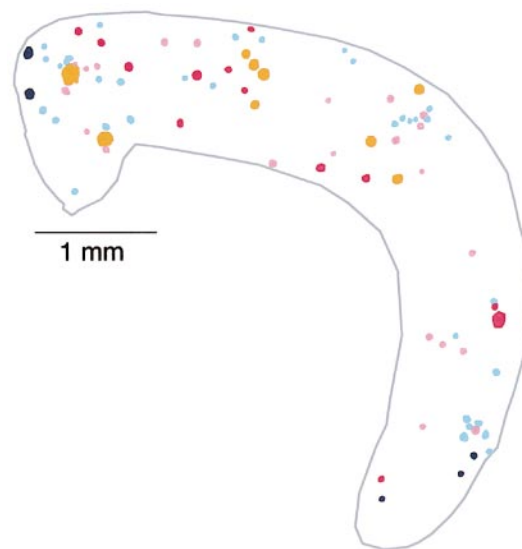
## DISCUSSION

These studies demonstrate the presence of neuritic dystrophy in NOS-containing cortical neurons in an animal model of AD and suggest that cerebral  $\beta$ -amyloid plaques give rise to the dystrophic change. The neuronal origin of the dystrophic diaphorase-positive structures is supported by their morphology (particularly the instances of continuity between normal-appearing axons and the dystrophic structures), by their neuron-specific contents (nNOS and somatostatin immunoreactivity), and by the absence of glial cell markers in the dystrophic structures. A causative role for  $\beta$ -amyloid in the genesis of this NOS-positive neuritic dystrophy is supported by the consistent presence of neuritic dystrophy in  $\beta$ -amyloid plaque-bearing animals, the consistent absence of neuritic dystrophy in age-matched wild-type animals, and the high degree of colocalization of  $\beta$ -amyloid plaques and NOS neuritic dystrophy. Even in young animals just beginning to develop plaques,  $\beta$ -amyloid deposits are frequently associated with NOS-positive neuritic dystrophy, and neuritic dystrophy is not seen without association with plaques. The neuritic dystrophy in these younger animals is not as morphologically complex as that seen in the older animals, suggesting that although the neuritic change is an early event after plaque deposition, the neuritic dystrophy continues to evolve over time.

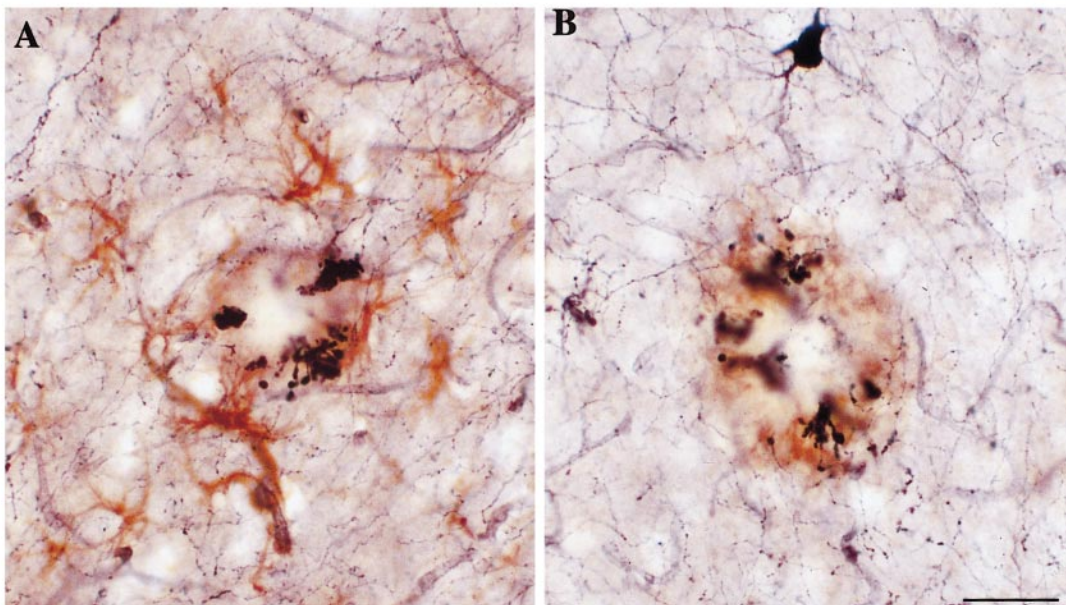
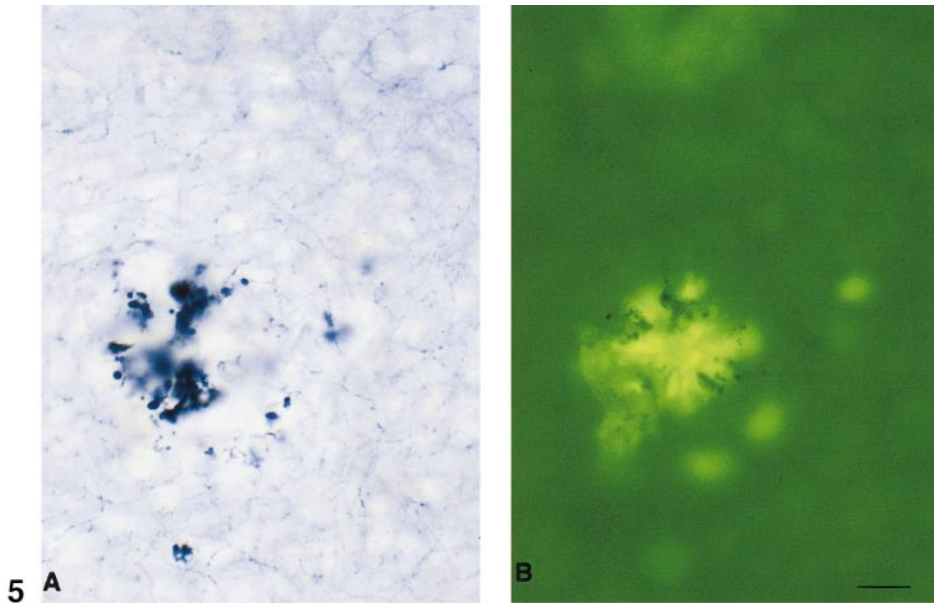
These findings support the  $\beta$ -amyloid cascade hypothesis in AD by demonstrating a robust neuronal effect of  $\beta$ -amyloid plaques. This observation is compatible with prior reports of unchanged neuron numbers in this strain (25) because those reports emphasized perikaryal numbers rather than neuritic changes. However, additional studies of other cortical neurotransmitters will be necessary in order to determine the degree to which the NOS-bearing neurons differ in  $\beta$ -amyloid sensitivity from other neurites in the vicinity.

Comparable studies with human tissue will also be necessary to establish the relevance of this animal model observation to the actual human disease. However, the literature on NOS-bearing neurons in human AD suggests that this neuronal population is vulnerable in AD. The well-established degeneration of somatostatinergic neurons (35) and depletion of cortical somatostatin in AD (2) are likely to reflect damage to the nNOS neuronal population, given the frequency of colocalization of these markers in cerebral cortex (51). The damage to NOS-bearing cortical neurons in AD is variously described as “a marked pruning and distortion of fiber plexuses with an apparent reduction in fiber density” but preserved cell number (26), atrophy of NOS-positive neurons (51), and outright loss of NOS-positive cortical neurons (37, 49, 57). Two of these studies (37, 57) also found that the NOS neuronal dropout was not seen in occipital cortex, an area less affected by the neurodegenerative process. We hypothesize that the variability in these pathological studies is due to a variability of disease stage in the specimens studied, and propose a model which reconciles our observations with the pathological literature.

We hypothesize that fibrillar  $\beta$ -amyloid induces an increase in nNOS in dystrophic neurites (as observed in the huAPP mice) at an early stage of the human disease. The toxic consequences of increased NO production might include subsequent death of the neurite, and eventually of the entire neuron, as seen in human brain. This model would predict a range of pathology



**FIG. 4.** Map of  $\beta$ -amyloid plaques in cerebral cortex of a 14-month-old mouse. Plaques are distinguished by their associated NOS-positive neuritic dystrophy (Fig. 4). Grade 0 neuritic dystrophy—dark blue. Grade 1 neuritic dystrophy—light blue. Grade 2 neuritic dystrophy—pink. Grade 3 neuritic dystrophy—red. Grade 4 neuritic dystrophy—yellow. NOS-positive neuritic dystrophy without associated plaque—green arrow.



**FIG. 5.** Double staining (for nitric oxide synthase and fibrillar  $\beta$ -amyloid) of a section of cerebral cortex of 14-month-old transgenic huAPP mouse. Nitric oxide synthase is labeled with diaphorase histochemistry (blue) and viewed in bright field (A), while fibrillar  $\beta$ -amyloid is labeled with thioflavin S (green) and viewed under fluorescence (B).

**FIG. 6.** Double staining of 14-month-old huAPP mouse brain for diaphorase (blue) and GFAP (A, brown) and diaphorase and MAC-1 (B, brown). Activated astrocytes (A) are seen surrounding the vicinity of the plaque-associated neuritic dystrophy. Activated microglia (B) are not as intensely stained and do not extend as far from the plaque as do the astrocytes. Neither astrocytes nor microglia appear to contribute to the dystrophic structures. Bar = 20  $\mu$ m.

depending on the “age” of the  $\beta$ -amyloid plaques, and thus reconcile the various observations noted in the pathology literature. The pathology in the transgenic mouse would be considered an example of very early Alzheimer pathology.

Several studies have directly examined the hypothesized interaction between  $\beta$ -amyloid and NOS. For example,  $\beta$ -amyloid induction of NOS has been described in cultured microglial cells (3, 16, 24), in

cultured astrocytes (1, 22, 41), and in both of these cell types *in vivo* after intrastriatal injection of  $\beta$ -amyloid (52). The effect of  $\beta$ -amyloid on neuronal NOS has been studied less extensively, but one study has noted the potential for  $\beta$ -amyloid to stimulate the release of NO in a neuronal cell line (23). An *in vivo* study of  $\beta$  amyloid toxicity revealed selective loss of nNOS neurons in rat striatum after  $\beta$ -amyloid injection (52).

Since nNOS enzyme activity is dependent on stimulation by calcium (5), it remains to be established whether the high levels of nNOS enzyme observed in the dystrophic neurites indeed produce elevated levels of NO. The literature on  $\beta$ -amyloid neurotoxicity and AD neuropathology, however, strongly suggests that NO may play a role in the progression of AD. Oxidative damage due to NO is thought to be mediated by the potent oxidant, peroxynitrite (9), which is generated from NO and superoxide anion. Peroxynitrite has been implicated in  $\beta$ -amyloid neurotoxicity *in vitro* (17, 32). There is also abundant evidence for increased levels of peroxynitrite-mediated damage (in the form of nitrotyrosine) in AD brain (15, 19, 45, 47).

In addition to the generation of oxidative damage, a variety of other possible mechanisms exist by which NO could alter brain function. Increased levels of NO might diffuse out of the affected neurite and account for some of the astrocytic and microglial response to the  $\beta$ -amyloid plaque. NO diffusion or signaling might even result in damage to other neuronal populations, such as the cholinergic neurites, which are known to make synaptic contacts with NOS neurons in rodent and human cortex (50). Increased NO production could also conceivably disturb cerebral blood flow or long-term potentiation, since NO has been shown to play a physiologic role in these processes (11, 18). The plausibility of these mechanisms depends on whether NO may result in abnormal signaling at some distance from the dystrophic neurite.

In summary, we have observed robust neuronal damage in an animal model of cerebral  $\beta$ -amyloidosis. The findings strengthen the  $\beta$ -amyloid cascade hypothesis of neurodegeneration in AD and support the validity of this transgenic mouse strain as an animal model of AD. The findings also suggest a possible mechanism in which NO mediates  $\beta$ -amyloid neurotoxicity in AD, though further investigations will be necessary to test the "NO hypothesis" and related hypotheses distinguishing oxidative, vascular, and signaling mechanisms. Such further investigations into mechanism may translate into new therapeutic strategies in AD.

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